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# 11-Hydroxysteroid Dehydrogenase type 1 is expressed in neutrophils and restrains an inflammatory response in male mice

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**Coutinho, Supplement**

## SUPPLEMENTARY FIGURES

***Supplementary Figure 1. Purity of mouse neutrophil preparations***

Peritoneal cells were incubated with Ly6G-phycoerythrin (PE) antibody followed by incubation with anti-PE secondary antibody magnetic beads. (A) Flow cytometry with Ly6G staining was used to quantify the purity of isolated neutrophils, which was generally ≥96%. (B) Neutrophil purity was confirmed by May-Geimsa staining of cytocentrifuged cells; left panel, total peritoneal cells prior to neutrophil isolation; centre panel, flow-through cells ($Ly6G^-$) and right panel, neutrophils ($Ly6G^+$ cells), images captured at x40 magnification.

***Supplementary Figure 2. 11β-HSD1 staining in peritoneal cells***

Flow cytometry was used to measure the number of 11β-$HSD1^+$ cells and to quantify levels of 11β-HSD1 (by mean fluorescence intensity, MFI). Representative dot plots of peritoneal cells collected 4h after thioglycollate injection (from 7-8 mice; $5x10^5$ cells/sample), showing 11β-$HSD1^+$ cells after incubation with 11β-HSD1 antibody (left panel), control IgG (centre panel) and cells incubated with secondary antibody only (right panel).

***Supplementary Figure 3. Monocyte numbers and 11β-HSD1 expression in monocytes from BM and blood during peritonitis***

Monocyte ($Ly6G^-$,$7/4^+$,$CD11b^+$) cell number was determined by flow cytometry, in freshly isolated (A) BM and (C) blood at various times following thioglycollate injection. 11β-HSD1 expression (mean fluorescence intensity, MFI) was measured in BM monocytes (B) and the following subsets of blood monocytes: (D) $7/4^{hi}$, (E) $7/4^{med}$ and (F) $7/4^{lo}$. Data are means ± SEM and were analysed by 1-way ANOVA followed by Dunnett's post-hoc test; *p<0.05, **p<0.01, ***p<0.001, n=6-8.

***Supplementary Figure 4. UE2316 inhibits 11β-HSD1 in peritoneal cells in vitro***

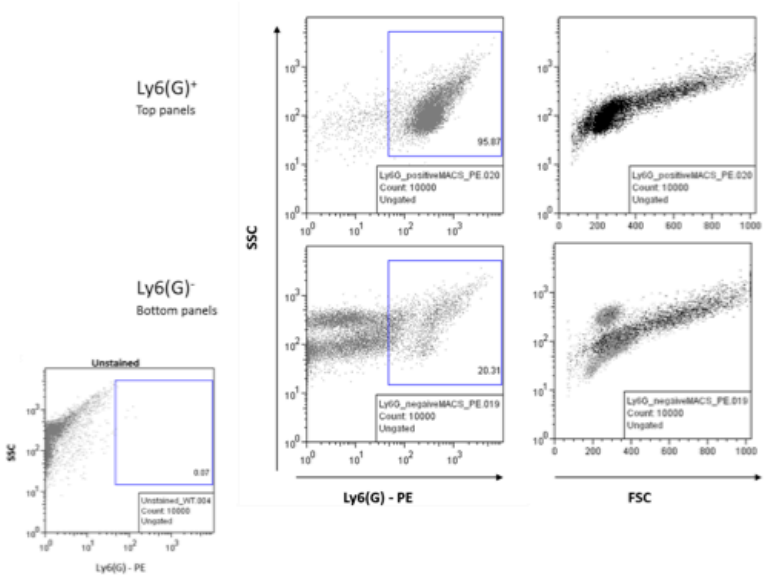
Peritoneal cells (2 x $10^5$) isolated 4h following thioglycollate injection were incubated with $^3$H-cortisone vehicle (solid line, circles) or 1μM UE2316 (dashed line, squares). Data are means of 2 samples each assayed in duplicate and show conversion (cpm) of $^3$H-cortisone to $^3$H-cortisol over time.

***Supplementary Figure 5. The effect of 11β-HSD1 inhibition upon CD11b expression in bone marrow and blood neutrophils during 4h peritonitis***

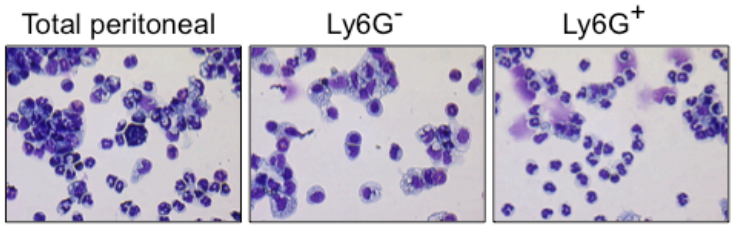
CD11b mean fluorescence intensity (MFI) was measured by flow cytometry performed on BM cells and blood collected from mice pretreated with vehicle (Veh, black bars) or UE2316 (white bars), 4h following injection of thioglycollate. (A) BM neutrophils, (B) blood neutrophils. Data are means ± SEM and were analysed by student's t-test, n=9-10.

Supplementary Figure 1

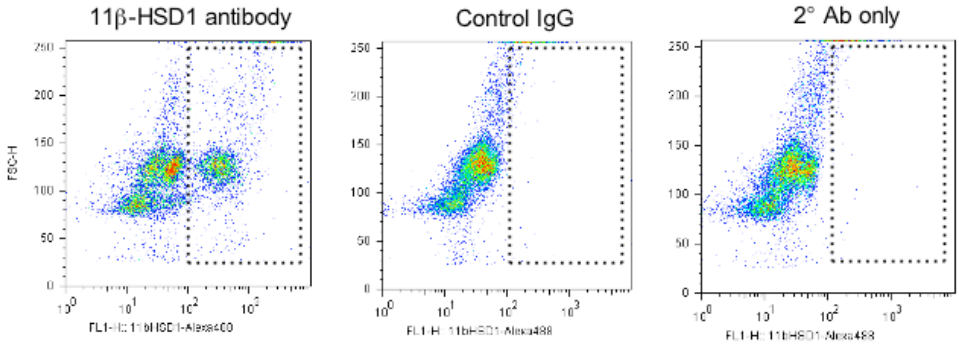
**A**



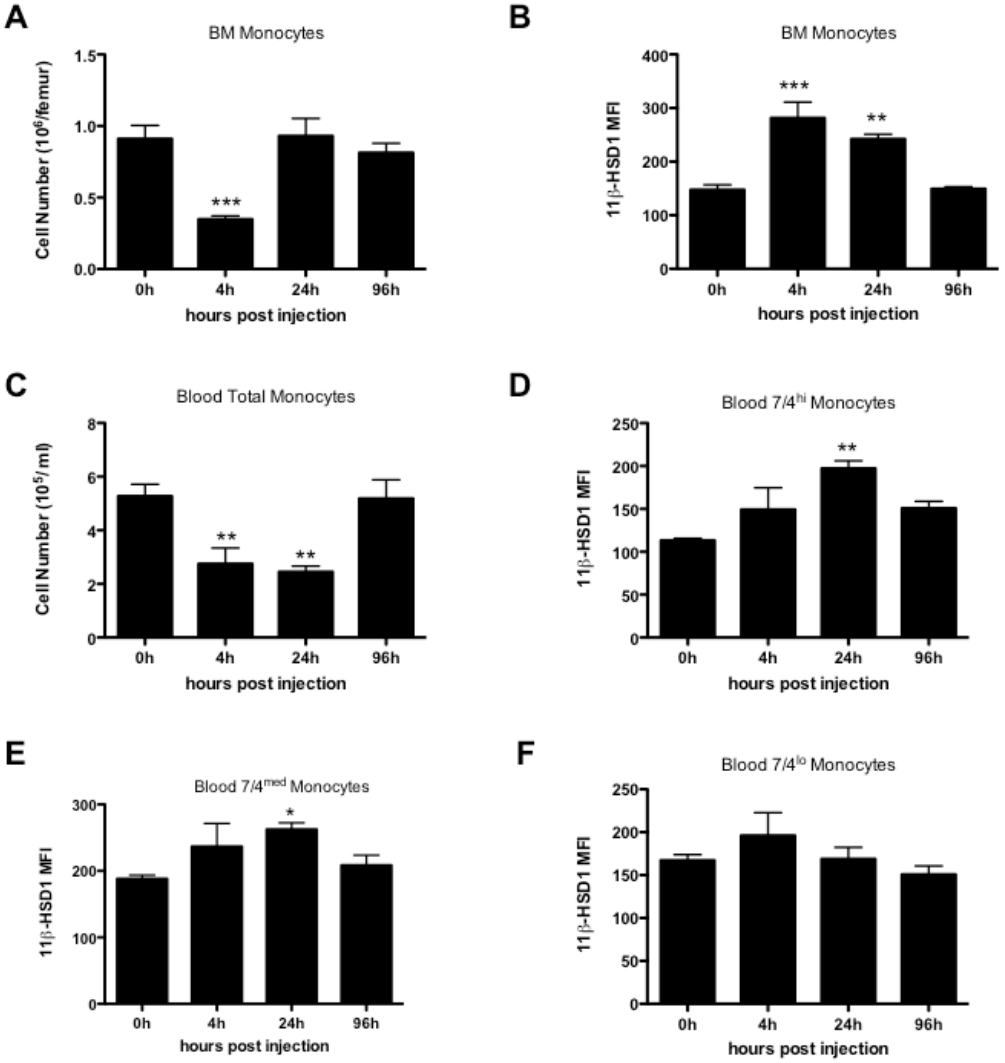

**B**



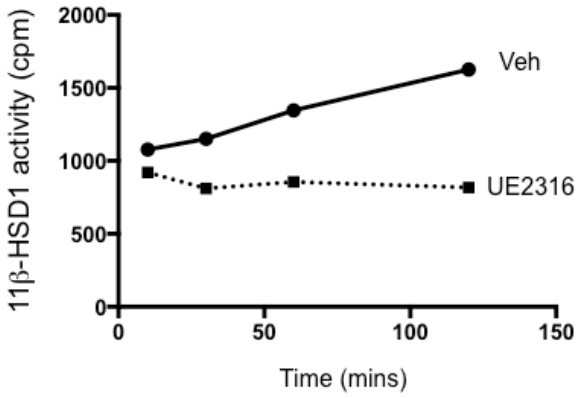
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5